Virus–host interactions: role of HIV proteins Vif, Tat, and Rev

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Introduction

The primary goal of a virus is the replication of its genome in an appropriate host cell and the production of progeny virions for the infection of new target cells. This effort is commonly met by antiviral responses by the host organism, which in most cases abolishes or limits virus infections. Viruses have developed different strategies to overcome these restrictions, some causing long-lasting chronic infections, others replicating in fast, lytic cycles. However, all viruses depend to a large degree on specific host factors, from the recognition of specific cell-surface receptors required for virus entry into a target cell to the packaging of cellular factors into virions.

HIV penetrates target cells through fusion with the host plasma membrane. This is followed by partial uncoating and reverse transcription of the viral RNA, and subsequent integration of the double-stranded complementary DNA into the host genome. The integrated provirus then serves as a template for the synthesis of viral proteins, which ultimately assemble into progeny virions that are released from the infected host cell. We are far from understanding all of the complex virus–cell interactions that take place during the HIV life cycle, however, our current knowledge suggests that such interactions occur at virtually every step of virus replication. The past few years have brought rapid progress in the identification and characterization of novel host factors supporting HIV replication. In particular, the recent identification of chemokine receptors as HIV co-receptors has significantly advanced our understanding of HIV cell tropism and entry. Less well defined, however, is the extent to which cellular factors are involved in post-entry events required for the establishment of a productive HIV infection.

Primate immunodeficiency viruses, including HIV-1, are characterized by the presence of a number of viral accessory genes that encompass *vif*, *vpr*, *vpx*, *vpu*, and *nef*. The *vif*, *vpr*, and *nef* genes are expressed in most HIV-1, HIV-2 and SIV isolates. In contrast, the *vpu* gene is found exclusively in HIV-1 and some SIV isolates. The *vpx* gene, on the other hand, is not found in HIV-1 isolates but is common to HIV-2 and most SIV isolates. Defects in accessory genes are frequently not correlated with a detectable impairment of virus replication in continuous cell lines, in contrast to primary cell types, which more closely reflect the in-vivo situation. However, it becomes increasingly clear that these proteins exert important functions in their relevant target cells *in vivo*, and most if not all of the HIV accessory proteins seem to exert multiple independent functions. For most of the HIV accessory and regulatory proteins the precise biochemical mechanisms are still under investigation, however, there is increasing evidence to suggest that none of the HIV accessory or regulatory proteins has catalytic activity on its own. Rather, they appear to function as adapter molecules that connect other viral or cellular factors to various cellular pathways. The goal of the current review is to summarize recent progress in the study of virus–host interactions involving the viral Tat, Rev, and Vif proteins.

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Vif: overcoming a cellular restriction to virus replication

Ever since its identification as a virus infectivity factor in 1987 [1,2], the *vif* gene, which initially was termed 'sor' or 'A' [2], was the subject of intense research. However, despite significant progress over the past 16 years, the biochemical function of Vif remains largely unclear. A number of studies have attempted to reveal the biochemical function of Vif, but have produced somewhate conflicting results, and many reported observations regarding Vif remain controversial. It is now generally accepted, however, that Vif-defective viruses are capable of entering target cells but encounter an early block in virus replication before integration of the viral genome into the host genome. It is also now generally accepted that Vif is packaged into viral particles, although its functional significance is still under investigation (Fig. 1, step 2). Nevertheless, a recent analysis of the molecular defect in Vif-defective HIV virions did not detect any apparent abnormalities in Vif-defective virions, except for the lack of Vif [3]. There has been significant discussion regarding the number of Vif molecules packaged into virions, with estimates ranging from less than one molecule per virion to as many as 100 molecules of Vif per virion. In general, studies employing virus from chronically infected cells or from stable cell lines reported lower amounts of virus-associated Vif than studies using virus from productively infected cells [4].

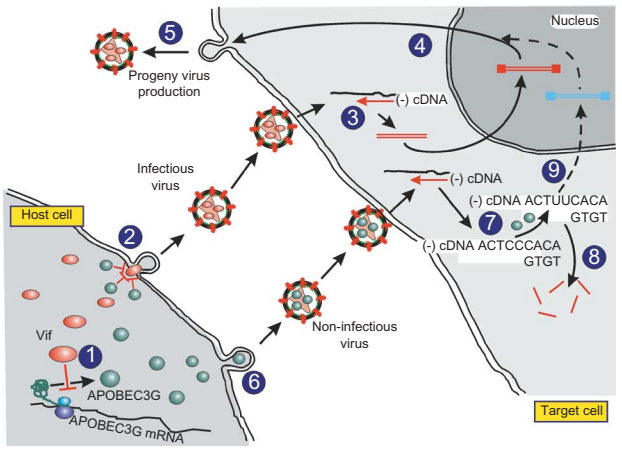

Fig. 1. Regulation of virus infectivity by Vif. Cells restrictive for the replication of Vif-defective HIV express the cytidine deaminase APOBEC3G. In the presence of Vif, intracellular de novo synthesis of APOBEC3G appears to be inhibited through a post-transcriptional mechanism (1). Vif is packaged into virions and associates with the viral nucleoprotein complex. Importantly, Vif inhibits the packaging of APOBEC3G into virions (2) thus increasing virus infectivity. Such viruses can enter a target cell (3), integrate into its host genome and produce infectious progeny virions (4,5). In contrast, the absence of Vif results in the packaging of APOBEC3G into virus particles (6). Such virions are capable of penetrating a target cell and initiate minus-strand cDNA synthesis [(−)-cDNA]. However, APOBEC3G causes hypermutation of the viral (−)-cDNA, resulting in the conversion of deoxycytidine to deoxyuridine (7). Deoxyuridine residues in the viral cDNA can be targeted by uracil-DNA glycosylase, which could lead to endonucleolytic cleavage by endonucleases present in the target cell (8). In an alternative model, hypermutated cDNA enters the nucleus (9), and integrates into the host genome but results in the production of defective or aberrant viral proteins (4). This can lead to an impairment of virus assembly or can result in the assembly of non-infectious viruses (5).

There is increasing evidence that the packaging of Vif into virus particles is functionally relevant. First, Vif packaging is specific and is mediated through an interaction with viral genomic RNA [5–7]. In addition, virus-associated Vif interacts with Gag or Gag–Pol precursor molecules [8] (K. Strebel, unpublished observation), and is stably associated with the viral nucleoprotein complex [5,9,10]. Finally, virus-associated Vif is proteolytically cleaved by the viral protease at a conserved sequence located near the C-terminus of the protein (residue 150 in HIV-1 Vif) [11]. Intravirion processing of Vif is not restricted to HIV-1 Vif, but was also observed for HIV-2 and some SIV Vif variants (K. Strebel, unpublished observation). Of note is the fact that mutations at or near the processing site that affect Vif processing were also found to affect Vif function, whereas mutations that did not affect Vif processing did not affect Vif function [11]. Interestingly, Vif processing separates the more conserved N-terminus of Vif from its highly variable C-terminal domain, which presumably contains a multimerization domain [12,13] as well as the immunodominant epitope. Removal of the C-terminal region of Vif induces a conformational change in Vif (K. Strebel, unpublished observation) that may expose new functional domains in the protein.

Vif is a highly insoluble protein with a strong tendency to aggregate. This characteristic has hampered efforts to purify Vif protein for structural analyses. Therefore, in contrast to most other HIV-encoded proteins, there are currently no structural data available for Vif. Attempts to define functional domains in Vif through biochemical analyses have demonstrated that residues throughout the protein are important for Vif function [14,15]. The only exception appears to be the very C-terminal region of Vif, as evidenced by the fact that a natural variant lacking the C-terminal 19 amino acids was found to be biologically active [16]. These results suggest that Vif contains multiple functional domains that might be important for the interaction with viral or cellular proteins. It is tempting to speculate that Vif, like most other HIV regulatory and accessory proteins, functions as a molecular adapter to connect otherwise unrelated viral or cellular mechanisms. However, the functionally relevant ligands of Vif have not yet been

defined. Also, the search for negative trans-dominant Vif variants, which would support such a model, has so far been unsuccessful [17].

Vif functions in a host cell-specific manner. Accordingly, Vif-defective viruses produced in permissive host cells are unrestricted and are thus capable of infecting both permissive and non-permissive target cells. In contrast, Vif-defective viruses grown in non-permissive host cells were unable to infect permissive and non-permissive target cells alike [18–24]. This suggests that host factors play a significant role in restricting virus replication. A number of host factors have been identified as possible targets of Vif. These include vimentin [10], HP68 [25], Hck [26], sp140 [27], and CEM15 [28]. Importantly, the expression of Hck and CEM15 appeared to be associated with the inhibition of viral infectivity in a Vif-dependent manner [26,28]. However, only CEM15 expression was closely linked to non-permissive cellular phenotypes, and, unlike Hck, did not seem to have additional effects on virus production. CEM15 thus represents to date the most promising factor that fits most, if not all, of the characteristics required of a protein associated with Vif-dependent host cell restriction: it appears to be expressed exclusively in non-permissive cells, and furthermore, expression in permissive cells was found to inhibit virus infectivity in the absence but not in the presence of Vif [28,29].

CEM15 is identical to APOBEC3G and is a member of the family of cytidine deaminases [30]. APOBEC3G was found to have DNA cytidine deaminase activity *in vitro* [31]. The physiological substrates of APOBEC3G are not currently known; however, the tissue-specific expression of APOBEC3G suggests a role in growth or cell cycle control [30]. Interestingly, mutations in the catalytic site of APOBEC3G were associated both with decreased cytidine deaminase activity and a loss of the inhibitory activity of APOBEC3G on HIV replication [32,33]. Most recently, four research groups almost simultaneously reported that APOBEC3G induces the hypermutation of newly synthesized HIV DNA [32–35], thus providing a plausible explanation for the antiviral activity of APOBEC3G. All four reports noted a significant increase in G to A mutations in the viral genome (Fig. 1, step 6). As APOBEC3G-induced C to U mutations will result in a G to A mutation on the complementary strand, the observed G to A changes are most consistent with hypermutation of the minus-strand DNA rather than viral genomic RNA. Consistent with this, a direct analysis of RNA from Vif-defective virions produced in non-permissive H9 cells without endogenous reverse transcription did not reveal G to A hypermutation, whereas an analysis of cDNA derived by endogenous reverse transcription of the same viruses revealed G to A hypermutation [32,35]. This suggests that all the factors involved in hypermutation of HIV cDNA are present in virions from non-permissive cells. Consistent with these results, APOBEC3G was found to be packaged into HIV-1 virions [28,29,36]. Details of how APOBEC3G interferes with the replication of Vif-defective HIV have yet to be investigated. However, the observation that APOBEC3G induced defects in Vif-defective virions that became more and more severe with each step of virus replication has led to the proposal that the APOBEC3G-induced inhibition of HIV replication is the cumulative result of multiple defects [33]. It is possible that hypermutation of proviral DNA induces aberrant stop codons or mutates viral proteins (Fig. 1, step 9). More likely, however, seems to be the possibility that deaminated minus strand DNA is targeted by uracil-DNA glycosylase, which could result in the degradation of viral DNA via a uracil-based excision pathway [32,34], and could thus lead to abortive infection typical of Vif-defective viruses (Fig. 1, step 8).

The mechanism by which Vif prevents hypermutation by APOBEC3G is still under investigation. Data from several groups, including our own, suggested that Vif inhibits the packaging of APOBEC3G into HIV-1 particles in a dose-dependent manner [29,36] (Fig. 1, step 2). Moreover, the inhibition of APOBEC3G packaging requires biologically active Vif protein, whereas a series of biologically inactive Vif variants, including point mutants and in-frame deletions of larger portions of Vif, did not affect APOBEC3G packaging (Fig. 1, step 6). The inhibition of APOBEC3G packaging by wild-type Vif protein is paralleled by a reduction in the cell-associated expression levels of APOBEC3G protein [29]. Interestingly, Vif did not affect the expression level of APOBEC3G messenger RNA [28,29], suggesting that Vif affects APOBEC3G protein expression via a post-transcriptional mechanism (Fig. 1, step 1). A major focus of future research will undoubtedly be how Vif blocks APOBEC3G-induced deamination of viral cDNA. This should lead to a detailed understanding of Vif function, and may reveal novel targets for antiviral therapy.

Tat: making the connections

Because of its crucial role in activating viral gene transcription, the HIV Tat protein has been a key focus of HIV research for many years. It is now well accepted that Tat functions as a molecular adapter, directing components of the cellular transcription machinery to the viral RNA to promote the processivity of transcription by the RNA polymerase II complex. Tat is a small protein of 101 amino acids that is expressed from a multiply spliced RNA early during HIV replication.

Tat contains several functional domains: residues 1–47 encompass the activation domain or co-factor-binding domain, whereas the basic domain located between residues 48 and 60 is required for RNA binding as well as the nuclear transport of Tat [37]. In addition, the C-terminal domain of Tat has been implicated in stimulating the co-transcriptional capping of HIV-1 mRNA through a direct interaction with the capping enzyme MceI [38].

In the absence of Tat, transcription from the HIV-1 long-terminal repeat (LTR) produces predominantly short, non-polyadenylated RNA that include the trans-activation response region (TAR) stem-loop structure. In contrast, the expression of Tat results in the production of longer, polyadenylated RNA and in increased gene expression [39–42]. The predominance of short transcripts in the absence of Tat is most likely caused by the poor processivity of RNA polymerase II transcription complexes recruited to the viral LTR and not, as was initially proposed, caused by an anti-terminator activity of TAR (Fig. 2b, step 1). There is a complex interplay between the positive transcription elongation factor b (P-TEFb) and negative transcription elongation factors 5, 6-dichloro-1-beta-D-ribofurano-sylbenzimidazole sensitivity-inducing factor and the negative elongation factor complex [43,44]. A key factor in the regulation of RNA polymerase II is the phosphorylation status of its carboxyterminal domain (CTD). Hypophosphorylation of the CTD correlates with low processivity of the RNA polymerase II complex, whereas hyperphosphorylation of the CTD promotes the processivity of the enzyme complex [45]. Phosphorylation of the CTD is regulated by P-TEFb containing a CTD-specific kinase activity (Fig. 2b, step 3). The nuclear Tat-associated kinase [46], which was originally identified as the kinase subunit of the P-TEFb complex [47,48], was subsequently identified as the cyclin-dependent kinase, CDK9 [49]. CDK9 can interact with different cyclin partners, including cyclin T1, cyclin T2a, cyclin T2b, and cyclin K [50]. However, Tat was found to recruit cyclin T1 selectively into the Tat–P-TEFb complex in the process of transcriptional activation from the HIV LTR promoter [51] (Fig. 2b, step 2).

Unlike most transcriptional activators, Tat does not bind to a DNA target but interacts with the TAR element, an RNA structure located near the 5'-end of the viral genome [37]. The TAR structure is an unusual stem-loop structure containing a three-nucleotide bulge (residues 23–25) and a six-nucleotide loop (residues 30–35) (Fig. 2a). Originally, the TAR RNA structure was mapped to residues 1–80 on the viral RNA, however, the minimal sequence element required for Tat-responsiveness was subsequently narrowed down to residues 19–42 (Fig. 2a) [52–54]. The bulge structure in TAR is essential for the high-affinity

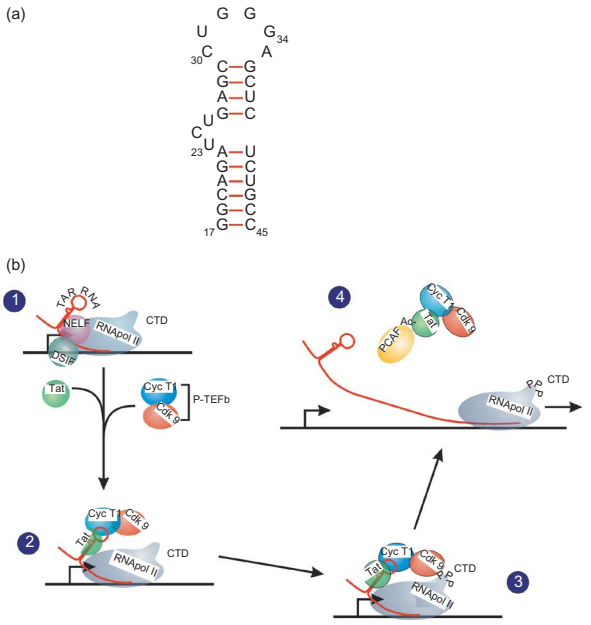

Fig. 2. (a) Structure of trans-activation response region RNA. Nucleotide residues are referred to in the text. (b) Transcriptional activation by Tat. A key factor in the regulation of transcription from the HIV-long-terminal repeat (LTR) promoter by RNA polymerase II (RNApol II) is the phosphorylation status of its carboxyterminal domain (CTD). Hypophosphorylation of the CTD correlates with low processivity, whereas hyperphosphorylation increases the processivity of the enzyme complex. In the absence of Tat, transcription from the HIV-1 LTR produces predominantly short RNA as a result of the hypophosphorylated state of RNApol II and the activity of 5, 6-dichloro-1-beta-D-ribofuranosylbenzimidazole sensitivity-inducing factor (DSIF) and negative elongation factor complex (NELF), which bind to hypophosphorylated RNApol II and inhibit transcriptional elongation (1). One of the kinases involved in CTD phosphorylation is Cdk9, which together with cyclin T1 constitutes the positive transcription elongation factor b (P-TEFb). Tat binds to the trans-activation response region (TAR) structure on the viral RNA and recruits P-TEFb through binding to cyclin T1 (2). Recruitment of P-TEFb to TAR induces hyperphosphorylation of CTD by Cdk9 and results in the dissociation of DSIF and NELF (3). Acetylation of Tat at Lys 50 creates a binding site for p300/CREB binding protein-associated factor (PCAF) and promotes the formation of a ternary complex of Tat-PCAF and P-TEFb. The interaction of PCAF with acetylated Tat was found to compete against TAR RNA binding of Lys50-acetylated Tat, and causes its dissociation from TAR RNA, thereby enhancing the transcriptional elongation of HIV-1 (4).

binding of Tat [55]. Earlier reports demonstrated that mutations in the TAR loop of HIV-1 did not interfere with Tat binding, but significantly reduced Tat transactivation [55,56]. This suggested that the TAR loop acts as a binding site for transcriptional co-factors. The search for TAR loop binding proteins led to the identification of cyclin T1. Unlike Tat, cyclin T1 alone

does not bind to TAR RNA. However, the interaction of Tat with cyclin T strongly enhances the affinity and specificity of the Tat–TAR RNA interaction [57]. In addition to the TAR bulge, at least one residue in the TAR loop, G34, is critical for the binding of the cyclin T1–Tat complex. G34 forms a base pair with residue C30 of the TAR loop [58], creating a single-nucleotide bulge at position 35 that is important for the overall structure of the TAR element [58]. The binding of cyclin T1 to Tat is zinc-dependent and involves a cysteine at position 261 in human cyclin T1. None of the other cysteine residues in human cyclin T1 are involved in Tat binding [59]. In the Tat–TAR–cyclin T1 complex, residues 252–260 of cyclin T1 form a Tat-TAR recognition motif that interacts with one side of the TAR RNA loop and enhances the interaction of Tat lysine-50 to the other side of the loop [58]. The TRM region of cyclin T1, when fused to Tat was found to be sufficient for high-affinity binding to the TAR recognition motif, and supported Tat transactivation in murine cells [59]. Interestingly, in murine cyclin T1, which does not support Tat transactivation [51,60–63], residue 261 is a tyrosine. Whereas murine cyclin T1 can bind Tat with similar efficiency to human cyclin T1, it is unable to form a functional P-TEFb–Tat–TAR complex [61,62]. This defect may explain the reported defect in HIV-1 transcription in rodent cells [51,57,61,64].

P-TEFb plays an important role in the regulation of mammalian gene expression. In that context, P-TEFb can function independent of TAR or Tat. This raised the question of why P-TEFb cannot activate the HIV-1 promoter in the absence of Tat and TAR. Previous data showed that TAR is only one component of the Tat-responsive target. Efficient Tat transactivation was observed only when TAR was present in conjunction with the HIV-1 LTR nuclear factor kappa B/SP1 DNA sequences [65,66]. Furthermore, Tat was able to mediate transcriptional activation *in vitro* through its interaction with Sp1 [67]. It was therefore proposed that TAR is required for Tat to stimulate the efficiency of elongation by RNA polymerase II, whereas Sp1 and other DNA sequence-specific transcription factors activate the rate of transcription initiation from the HIV LTR promoter [39]. Consistent with this idea is the observation that both murine and human cyclin T1 are able to interact with SP1 to allow Tat/TAR independent transcription, and that SP1 is necessary to recruit cyclin T1 to the HIV-1 LTR [68]. Finally, it was shown that the Tat-mediated assembly of active transcription complexes is regulated by nuclear bodies through modulation of the availability of cyclin T1 and other co-factors at the site of transcription [69]. This is supported by biochemical and biophysical analyses, which suggest that cyclin T1 interacts with the promyelocytic leukemia (PML) protein *in vivo* in PML nuclear bodies [69].

Apart from its function in promoting the processivity of RNA polymerase II, Tat has a function in remodeling chromatin near the transcription start site. Integrated proviral DNA is incorporated into the cellular chromatin and covered at specific sequences with nucleosomes [70]. Tat-mediated chromatin remodeling involves the inhibition of cellular histone acetylases, such as p300/CREB-binding protein (CBP), p300/CREB binding protein-associated factor (PCAF), and TIP60 [71–74]. In the case of CBP, Tat was found to induce substrate selectivity and to inhibit the acetylation of histones by CBP severely. No effect was seen on the basal level acetylation of other substrates, such as p53 and MyoD [75], whereas ultraviolet-induced acetylation of p53 was severely inhibited in HIV-infected cells [76]. However, the ultraviolet-induced inhibition of p53 acetylation by HIV infection was not strictly correlated with the levels of Tat, suggesting that other factors (e.g. Nef) might be involved in the stress-induced p53 response [76]. Apart from its negative effect on histone acetylation, Tat was found to promote acetylation of the p50 subunit of nuclear factor kappa B by p300/CBP, adding further evidence for the ability of Tat to alter the substrate specificity of p300/CBP [77].

Tat itself was found to be a substrate for acetylation by p300/CBP and PCAF [78–81]. Acetylation was found at lysine residues at positions 28, 50 and 51 [80]. As lysines 50 and 51 of Tat are located in its RNA binding motif, acetylation of these residues could regulate the association of Tat and TAR or affect the stability of Tat–TAR–cyclin T1 complexes. A mutation of lysines 50 and 51 in Tat was found to inhibit acetylation at these sites, and significantly reduced Tat transactivation [80]. The functional importance of Tat-acetylation is still under investigation, however, it is possible that the acetylation of Tat affects its three-dimensional structure and could create or expose new protein binding domains. Consistent with such a mechanism is the recent observation that lysine 50-acetylated Tat can bind to the transcriptional co-activator PCAF [81]. Such an interaction of PCAF with acetylated Tat was found to compete against the TAR RNA binding of lysine-acetylated Tat [82]. These data are consistent with the model that Lys50 acetylation of Tat causes its dissociation from TAR RNA, thereby enhancing the transcriptional elongation of HIV-1 [79,80] (Fig. 2b, step 4).

Apart from its crucial role in activating the transcription of the HIV genome, Tat was associated with a number of additional activities. Because of its unusual ability to exit HIV-infected cells and enter uninfected bystander cells, Tat has been investigated for its potential effect on bystander cells. Extracellular Tat was found to induce the production of cytokines such as transforming growth factor beta, IL-2, or IL-6 [83–86], cause

neurotoxicity in the central nervous system [87–93] and apoptosis in cultured peripheral blood mononuclear cells and at least one CD4 T-cell line [94–97]. On the other hand, Tat was found to upregulate the anti-apoptotic gene Bcl-2 in infected primary human macrophages, suggesting that in certain cell types, Tat expression may contribute to cell survival [98]. Some of these effects may be caused by an interaction of extracellular Tat with specific cell-surface receptors that trigger the activation of cellular signal transduction pathways. However, some of these effects may also be caused by Tat after internalization into uninfected bystander cells. Tat was found to bind to tubulin and polymerized microtubules in the cytoplasm of Jurkat cells, altering microtubule dynamics and activating a mitochondria-dependent apoptotic pathway [99]. Last but not least, the two-exon form of Tat was found to suppress reverse transcriptase activity during the late stages of viral replication and to increase viral infectivity, presumably by preventing the premature synthesis of viral DNA [100]. Finally, Tat was found to exhibit RNA annealing activity and to promote the placement of transfer RNA onto viral RNA, although the significance of this observation for the in-vivo function of Tat remains to be investigated [101].

Rev: shuttling viral RNA

Transcription in HIV takes place from a single promoter located within the 5'-LTR. The resulting full-length primary transcript functions both as genomic RNA and as mRNA for the expression of the *gag* and *pol* genes. Expression of genes downstream of *gag/pol*, however, requires extensive splicing of the primary transcript, resulting in a complex mixture of singly or multiply spliced RNA. Unlike the fully spliced mRNA species encoding Tat, Rev, and Nef, which are readily transported to the cytoplasm, the export of unspliced or partly spliced transcripts requires the activity of Rev. The Rev protein contains an arginine-rich RNA binding motif that binds to a stem-loop structure, known as the Rev response element (RRE), located in the env gene [102] (Fig. 3, step 1). The same arginine-rich motif in Rev also acts as a nuclear localization signal (NLS), which is required for the transport of Rev from the cytoplasm to the nucleus. The Rev NLS promotes the direct binding of the protein to the nuclear import factor importin, which targets the resultant protein complex to the nucleus [103] (Fig. 3, step 2). In addition, the nucleolar phosphoprotein B23, a putative ribosome assembly factor with affinity to NLS-containing proteins, was found to play a role in the nuclear import of Rev [104]. After transport to the nucleus, the formation of multimeric complexes between Rev and its RRE-containing target RNA is thought to displace B23 from Rev and to mask the

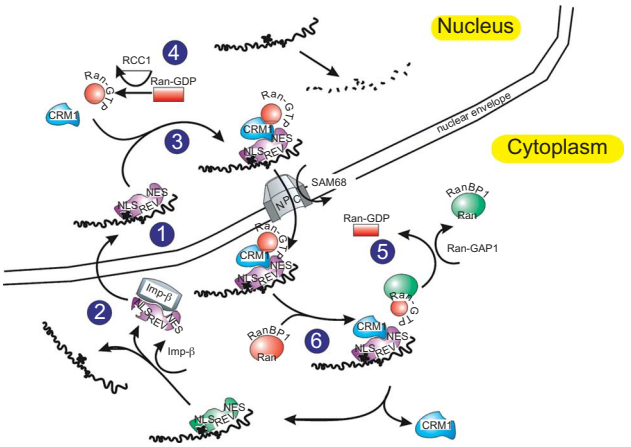

Fig. 3. Regulation of nuclear export of viral RNA by Rev. After synthesis in the cytoplasm, Rev is rapidly transported to the nucleus through an interaction of its arginine-rich nuclear localization signal (NLS) with the nuclear import factor importin β (Imp-β) (2). The same arginine-rich motif in Rev is responsible for binding to the Rev response element (RRE), located in the env gene (1). After transport to the nucleus, the formation of multimeric complexes between Rev and its RRE-containing target RNA is thought to mask the NLS and expose a nuclear export signal that mediates the interaction of Rev with nuclear export factors. The nuclear export of Rev–RRE complexes involves Crm1 (3). Human Crm1 localizes to the nuclear pore complex and the nucleoplasm and interacts with nuclear pore proteins. The association of Crm1 with Rev is thought to be regulated by Ran, a cellular guanosine triphosphate (GTP)ase. In the nucleus, the chromatin-bound exchange factor RCC1 generates Ran-GTP (4), whereas in the cytoplasm Ran-GTP is converted into Ran-guanosine diphosphate through RanGAP1, a cellular GTPase activating protein, resulting in a Ran-GTP gradient across the nuclear membrane (5). The binding of RanBP1 to Ran-GTP appears to be the key in catalysing the disassembly of Crm1–Rev–Ran-GTP complexes in the cytoplasm (6). Ran-GTP associated with RanBP is subsequently subjected to GTP hydrolysis, thus preventing Crm1 from rebinding Ran-GTP. This results in the release of Crm1 from Rev/RRE complexes.

NLS [105,106]. The multimerization of Rev on the RRE is initiated presumably by the high-affinity binding of the first Rev monomer to the Rev binding element in the RRE structure, followed by the cooperative binding of up to 12 additional Rev monomers to the RRE region [107–109]. This cooperative assembly of Rev on the RRE is thought to be accomplished via a series of symmetrical tail-to-tail and head-to-head protein–protein interactions [110].

The nuclear export of Rev–RRE complexes requires a nuclear export signal (NES) that mediates the interaction of Rev with nuclear export factors. The Rev NES is located in the C-terminal half of Rev, and consists of a leucine-rich stretch of amino acid residues [111,112]. The nuclear export of Rev–RRE com-

plexes involves Crm1, an evolutionary conserved 110 000 M_r protein that acts as a cellular receptor for NES-containing proteins [113–115] (Fig. 3, step 3). Human Crm1 localizes to the nuclear pore complex (NPC) and the nucleoplasm and interacts with nuclear pore proteins [116,117]. Crm1 was found to interact with Rev–RRE complexes containing an intact Rev NES but not with the M10 mutant of Rev containing an inactive NES [118]. The association of Crm1 with NES-containing proteins such as Rev is thought to be regulated by Ran, a cellular guanosine triphosphate (GTP)ase [113,115,119]. In the nucleus, the chromatin-bound exchange factor RCC1 generates Ran-GTP, which is the GTP-bound form of Ran (Fig. 3, step 4), whereas in the cytoplasm Ran-GTP is converted into Ran-guanosine diphosphate through RanGAP1, a cellular GTPase-activating protein, resulting in a Ran-GTP gradient across the nuclear membrane [119] (Fig. 3, step 5). It is believed that this asymmetric distribution of Ran-GTP plays a crucial role in nuclear import and export. High nuclear Ran-GTP levels favor the cooperative binding of Ran-GTP and NES-containing proteins such as Rev to Crm1 [113]. Such complexes are kinetically very stable, and their disassembly in the cytoplasm requires an additional factor, RanBP1 [120]. The binding of RanBP1 to Ran-GTP appears to be the key in catalysing the disassembly of the Crm1–Rev–Ran-GTP complexes (Fig. 3, step 6). Ran-GTP associated with RanBP is subsequently subjected to GTP hydrolysis, thus preventing Crm1 from rebinding Ran-GTP [120]. This results in the release of Crm1 from the Rev–RRE complexes.

In addition to Crm1, the nuclear export of Rev–RRE complexes was found to require the eukaryotic initiation factor (eIF-5A) [121]. The importance of eIF-5A in the nuclear export of the Rev–RRE complexes is highlighted by the fact that two non-functional mutants of eIF-5A, still capable of interacting with Rev–RRE complexes, were found to block Rev-mediated nuclear export when constitutively expressed in human CEM T cells [122]. In addition, microinjection experiments in somatic cells confirmed the crucial role of eIF-5A in nuclear export [121]. EIF-5A was found to be localized at the nucleoplasmic face of the NPC, and to interact specifically with nucleoporins CAN/nup214, nup153, nup98 and nup62, which are involved in nuclear export [123]. The precise role of eIF-5A in Rev function remains to be defined; however, it was proposed that eIF-5A may act as an adapter that targets the Rev-NES to the nucleoplasmic face of the NPC, and mediates efficient binding to CRM1 [123].

Another host factor implicated in Rev nuclear export is SAM68. SAM68 was originally described as a 68 000 Mr Src-associated protein in mitosis [124], and was found to promote the nuclear export of Rev in astrocytes [125]. Using an antisense expression strategy it was subsequently shown that the downmodulation of endogenous Sam68 in 293T and Jurkat cells but also in peripheral blood mononuclear cells significantly inhibited HIV expression by inhibiting the CRM1-mediated export of nuclear Rev, resulting in the nuclear retention of both Rev and Crm1 [126].

In conclusion, much progress has been made in understanding the molecular mechanisms of HIV regulatory and accessory gene products. There is accumulating evidence to suggest that all of the HIV accessory and regulatory proteins share a common function as adapter molecules to recruit cellular factors for various steps in the viral replication cycle. In the case of Tat, the recruitment of a variety of transcription factors to nascent HIV RNA is crucial to promote the processivity of RNA polymerase II transcription. Rev on the other hand has the ability to shuttle between the cytoplasm and nucleus of HIV-infected cells through reversible binding to nuclear import and export factors. Nuclear Rev can binds to the RRE element on viral RNA promoting their export from the nucleus to the cytoplasm. Like Tat and Rev, Vif has the ability to bind specifically to viral RNA, and was found to interact with a variety of host factors. It is therefore possible that Vif similarly functions as a molecular adapter molecule. However, in contrast to Tat and Rev, whose interaction with the TAR and RRE structures, respectively, has been well established, the RNA motif recognized by Vif is currently not well defined. It also remains to be shown if and how the interaction of Vif with viral RNA, as well as viral and cellular proteins, is connected to the ability of Vif to regulate viral infectivity.

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